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Estimation of stream salamander (Plethodontidae, Desmognathinae and Plethodontinae) populations in Shenandoah National Park, Virginia, USA

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Stream salamanders in the family Plethodontidae constitute a large biomass in and near headwater streams in the eastern United States and are promising indicators of stream ecosystem health. Many studies of stream salamanders have relied on population indices based on counts rather than population estimates based on techniques such as capture-recapture and removal. Application of estimation procedures allows the calculation of detection probabilities (the proportion of total animals present that are detected during a survey) and their associated sampling error, and may be essential for determining salamander population sizes and trends. In 1999, we conducted capture-recapture and removal population estimation methods for *Desmognathus* salamanders at six streams in Shenandoah National Park, Virginia, USA. Removal sampling appeared more efficient and detection probabilities from removal data were higher than those from capture-recapture. During 2001-2004, we used removal estimation at eight streams in the park to assess the usefulness of this technique for long-term monitoring of stream salamanders. Removal detection probabilities ranged from 0.39 to 0.96 for *Desmognathus*, 0.27 to 0.89 for *Eurycea* and 0.27 to 0.75 for northern spring (*Gyrinophilus porphyriticus*) and northern red (*Pseudotriton ruber*) salamanders across stream transects. Detection probabilities did not differ across years for *Desmognathus* and *Eurycea*, but did differ among streams for *Desmognathus*. Population estimates of *Desmognathus* decreased between 2001-2002 and 2003-2004 which may be related to changes in stream flow conditions. Removal-based procedures may be a feasible approach for population estimation of salamanders, but field methods should be designed to meet the assumptions of the sampling procedures. New approaches to estimating stream salamander populations are discussed.

INTRODUCTION

Stream salamanders including *Desmognathus*, *Eurycea*, *Gyrinophilus* and *Pseudotriton* species in the family Plethodontidae (lungless salamanders) are found in and near seeps and streams in the eastern United States. These salamanders play an important role in nutrient cycling and energy flow and can be top vertebrate predators in fishless headwater streams (DAVIC, 2002). In Appalachian old growth forests, plethodontid salamanders are extremely abundant (up to 18,486 individuals/ha) and constitute a large biomass (16.53 kg/ha) exceeding that of birds (PETRANKA & MURRAY, 2001). Stream salamander larvae develop in seeps and streams. After transformation, juveniles and adults spend part of their life in the leaf litter, rocky substrate and banks along streams, foraging on the surface on wet or humid nights, and hiding beneath rocks, logs, leaves, moss, bark, and in burrows during the day (PETRANKA, 1998).

Because stream salamanders may serve as indicators of stream ecosystem health (CORN & BURY, 1989; PETRANKA et al., 1993; WELSH & OLLIVIER, 1998; SOUTHERLAND et al., 2004), identifying reliable survey methods and population estimation techniques for this group are important. Many studies of stream salamanders have used population indices such as raw counts and densities (WELSH & OLLIVIER, 1998; BARR & BABBITT, 2002), although studies using population estimates based on capture-recapture and removal sampling have also been conducted (BRUCE, 1995; NIJHUIS & KAPLAN, 1998; PETRANKA & MURRAY, 2001). Population estimates that include detection probabilities (the proportion of total animals present that are detected during a survey) and their associated sampling error may be essential in determining population sizes and trends.

Detection probabilities ($\hat{p}$) for salamanders can vary spatially, temporally, and by species and age class (JUNG et al., 2000; SALVIDIO, 2001; BAILEY et al., 2004). If $\hat{p}$'s differ among study sites or over time, population indices will not be comparable unless differences in detection are estimated during sampling. Also, magnitude of detection probability can influence precision and bias of estimated population sizes; if $\hat{p}$'s or recapture rates of animals are low, standard errors of estimated population sizes become large, leading to unreliable or biased estimates.

The purpose of this investigation was two-fold. A study conducted in 1999 was designed to compare capture-recapture and removal estimation methods for stream salamanders and determine which method was more efficient and provided higher detection probabilities. A study conducted from 2001-2004 was meant to assess the usefulness of the preferred technique (removal estimation) for long-term monitoring of stream salamanders in Shenandoah National Park, Virginia, USA.

MATERIALS AND METHODS

For all stream salamander surveys in Shenandoah National Park, we recorded salamander species, age class (those with gills were recorded as larvae and those without were classified as adults), and snout-vent length (SVL) and total length (in mm). Because larvae of some species were occasionally not distinguished in the field and/or because of low sample sizes, we combined data for northern dusky (*Desmognathus fuscus*) and seal (*D. monticola*) salamanders into *Desmognathus* and combined data for northern spring (*Gyrinophilus porphyriticus*) and northern red (*Pseudotriton ruber*) salamanders into *Gyrinophilus/Pseudotriton* for analyses. The northern two-lined salamander (*Eurycea bislineata*) is found in the northern part of the park, whereas the southern two-lined salamander (*E. cirrigera*) is found in the southern part (Ghitea & Satler, 1990). Herein, we treat the two species, which are indistinguishable morphologically, as one when streams throughout the park are considered. Stream salamander species in the park not detected in our surveys included the long-tailed (*E. longicauda*) and three-lined (*E. guttolineata*) salamanders (Witt, 1993).

In 1999, we compared capture-recapture and removal methods to estimate stream salamander populations at six streams in the park. Three of the streams were first order (Jeremy's Run, Land's Run, Piney River) and the other three were second order. Two of four observers turned over the top layer of rocks and other objects greater than 6.4 cm maximum width or length within two 50 × 1 m transects (one on each side of the stream channel, 100 m^2 total area) at each stream, sampling the terrestrial habitat immediately adjacent to the wetted stream channel. Larvae were captured in the hyporheic zone and comprised 9 ± 2.8 % (mean ± s_x) of *Desmognathus* observations, 35 ± 8.7 % of *E. bislineata* observations, and 68 ± 11.6 % of *Gyrinophilus/Pseudotriton* observations across all surveys.

Approximately weekly from 9 July to 13 August 1999, we captured salamanders during the day by hand and dipnet and batch marked larvae and adults of or above 25 mm SVL using visible implant fluorescent elastomer (VIE, Northwest Marine Technologies, Inc.), a biocompatible latex-based dye injected just under the skin. We used one of three VIE colors (orange, red, green) at one of four positions just behind the forelimbs or in front of the hindlimbs and checked marks under a blanket using an ultraviolet light (Jung et al., 2000). Other studies have shown that VIE marks are more permanent and cause salamanders less harm than toe-clipping (Davis & Ovaska, 2001; Marold, 2001). Identities and measurements of unmarked, marked and recaptured salamanders were recorded at each survey. The percent of salamanders that escaped per stream across capture-recapture surveys averaged 36 ± 2.2 % for *Desmognathus*, 38 ± 5.5 % for *E. bislineata*, and 43 ± 10.3 % for *Gyrinophilus/Pseudotriton*.

After completing 5-6 capture-recapture surveys (five at Keyser Run, Pass Run Tributary, Piney River), we conducted temporary removal sampling of stream salamanders from 23 to 28 August 1999 at the same transects. Three passes, at least two hours apart, were made each day for two consecutive days for a total of six removal passes. We tallied the number of larvae and adults of each species removed at each pass and kept species and age classes in separate buckets with stream water positioned in the stream in the shade. All salamanders were released back to transects after the final pass.

During June and July of 2001-2004, we used temporary removal sampling as above though with 2-3 passes conducted one after the other during the day at 1-2 transects at each of eight streams in the park. Five of the streams were first order and three were second order or higher. Surveys were also conducted at a ninth stream, Staunton River, but no salamanders were detected there, presumably due to residual effects of a large flood along this river in 1995

For these surveys, we used a modified transect design, transects were 15 m long and 2 m wide and located on only one side of the stream, spanning 1 m along the stream bank and 1 m in the stream channel. This design allowed for capture of significantly more larval *Eurycea* ($F = 21.9$, $df = 1$, $P < 0.001$) and *Gyrinophilus*/*Pseudotriton* ($F = 4.4$, $df = 1$, $P = 0.043$) salamanders compared to the 1999 stream transects, larvae comprised 5 ± 2.2 % of *Desmognathus* observations, 75 ± 4.1 % of *Eurycea* observations, and 89 ± 4.2 % of *Gyrinophilus*/*Pseudotriton* observations. Our summer surveys mostly missed *Desmognathus* larvae, which typically transform by June-July (PETRANKA, 1998).

For capture-recapture data from 1999, we estimated population sizes ($\hat{N}$) and detection probabilities ($\hat{p}$) and their standard errors (s_x) using maximum likelihood and Bayesian estimators. These models assume a closed population and fit a series of models that differ in their assumptions about variation in $\hat{p}$ during sampling (OTIS et al., 1978, REXSTAD & BURNHAM, 1991). The test for population closure in program CAPTURE showed that all populations at each stream were closed (all $P > 0.05$), so open population models were not used (REXSTAD & BURNHAM, 1991). At least one assumption of capture-recapture models, that all animals captured are marked, was not met because we estimated only a subset of the populations, i.e., individuals of or above 25 mm SVL. The Bayesian estimator (GAZEY & STALEY, 1986) assumes prior distributions for N and p and estimators are derived based on the posterior distribution. We used a uniform (0,1) prior distribution on p and a diffuse negative binomial prior distribution on N (GEORGE & ROBERT, 1992). We fit the Bayesian model using a Markov chain Monte Carlo technique known as Gibbs sampling, in which the posterior distributions are estimated by simulation. Bayesian estimators do not rely on the asymptotic properties of maximum likelihood estimators, and hence are preferred for small sample sizes (GAZEY & STALEY, 1986).

For removal data from 1999 and 2001-2004, we calculated population estimates using Zippin model M_B (ZIPPIN, 1958; WHITE et al., 1982), which assumes a behavioral response to capture. Escaped salamanders were excluded from removal pass counts. Escapes were higher in 1999 compared to 2001-2004, when the percent of salamanders that escaped per stream across all removal samples averaged 36 ± 3.9 % and 26 ± 3.1 % for *Desmognathus*, 43 ± 7.3 % and 15 ± 1.9 % for *Eurycea*, and 44 ± 18.7 % and 11 ± 3.0 % for *Gyrinophilus*/*Pseudotriton*, respectively. Basic assumptions for removal studies include population closure, equal sampling effort, equal catchability, and effective reduction of the population after each search. Unfortunately in the case of stream salamanders, some of these assumptions are difficult to meet (BRUCE, 1995).

We used the "closed captures" selection in program MARK (WHITE & BURNHAM, 1999) to calculate $\hat{N}$, $s_x(\hat{N})$, $\hat{p}$ and $s_x(\hat{p})$ for capture-recapture model M_0, which assumes a constant capture probability, and removal model M_B. For *Desmognathus* data in 1999, we used paired t tests to test for significant differences between the $\hat{p}$'s of the capture-recapture and removal estimates and used program CONTRAST (SAUER & WILLIAMS, 1989; HINES & SAUER, 1990) to test whether $\hat{p}$'s from the capture-recapture and removal data differed among streams. For the 2001-2004 data, we compared whether $\hat{p}$'s estimated using removal models differed among streams within years and among years within streams for *Desmognathus* and *Eurycea* using a Chi Square test implemented in program CONTRAST. Analyses were conducted within groups (streams or years), then pooled to provide a composite Chi Square test with summed degrees of freedom. We also tested for differences among years and streams in $\hat{N}$ for *Desmognathus* and *Eurycea* using a two-way ANOVA in SPSS (Norusis, 1992).

Results

Across the capture-recapture surveys at the six streams in 1999, we marked 180 *Desmognathus*, 62 *E. bislineata*, and 17 *Gyrinophilus/Pseudotriton* (tab. 1). Recapture rates were fairly low, ranging from 0 to 33 % across species and stream sites (tab. 1). Because the numbers of marked and recaptured salamanders were low, the simplest model (model M_O), which assumes a constant capture probability, was usually the model of choice in program CAPTURE; the results of this model are presented in tab. 2. Unless a population is large or exhibits high capture probabilities, model selection may not be able to detect a pattern in $\hat{p}$'s and will select the default model M_O (Menkens & Anderson, 1988). Because capture-recapture estimates based on maximum-likelihood and Bayesian models were from the same data set, values for $\hat{N}$ and $\hat{p}$ from these methods were quite similar; we used the estimates based on maximum-likelihood for analyses. We were only able to calculate capture-recapture population estimates for *E. bislineata* and *Gyrinophilus/Pseudotriton* at one stream each, Jeremy's Run, where one individual of each species was recaptured (tab. 2). *Desmognathus* individuals were recaptured at all 6 streams and capture-recapture $\hat{p}$'s averaged 0.06 ± 0.014 (range: 0.02-0.10) (tab. 2). Capture-recapture $\hat{p}$'s differed among streams for *Desmognathus* ($\chi^2 = 13.3$, $df = 5$, $P = 0.02$). Capture-recapture does not perform well unless $\hat{p}$'s exceed 0.30 (White et al., 1982), which was never the case. Because of this, the standard errors of $\hat{N}$ were sometimes very large (tab. 2).

Based on the six-pass removal data from 1999, population estimates could be calculated at all 6 stream transects for *Desmognathus*, 3 for *E. bislineata*, and 2 for *Gyrinophilus/Pseudotriton* (tab. 2). Removal $\hat{p}$'s averaged 0.25 ± 0.077 (range: 0.08-0.61) for *Desmognathus* across stream transects, 0.25 ± 0.079 (0.09-0.35) for *E. bislineata*, and 0.44 ± 0.060 (0.38-0.50) for *Gyrinophilus/Pseudotriton*. Removal $\hat{p}$'s differed among streams for *Desmognathus* ($\chi^2 = 27.9$, $df = 5$, $P < 0.001$). Removal $\hat{p}$'s were significantly higher than those based on capture-recapture for *Desmognathus* ($t = 2.2$, $df = 5$, $P = 0.04$, tab. 2).

For the 2001-2004 data, we calculated removal population estimates for stream salamanders at stream transects at the eight streams (tab. 3). Estimation was not possible when zero counts occurred on the second pass when two passes were used or on the second and third passes when three passes were used, or when counts increased across subsequent passes. We could calculate population estimates at 55 %, 54 % and 13 % of the total 56 stream transects surveyed from 2001 to 2004 for *Desmognathus*, *Eurycea* and *Gyrinophilus/Pseudotriton*, respectively (tab. 3). When estimates were calculable, $\hat{p}$'s averaged 0.66 ± 0.025 (range 0.39-0.96) for *Desmognathus* across stream transects, 0.64 ± 0.031 (0.27-0.89) for *Eurycea*, and 0.62 ± 0.065 (0.27-0.75) for *Gyrinophilus/Pseudotriton* (tab. 3). These detection probabilities were much higher than those found in 1999. Using program CONTRAST, we found that $\hat{p}$'s differed among streams for *Desmognathus* ($\chi^2 = 29.7$, $df = 17$, $P = 0.028$) but not for *Eurycea*. Detection probabilities did not differ among years at a stream for either *Desmognathus* or *Eurycea*.

Table 1. – Numbers of salamanders summed across 5 to 6 capture-recapture surveys in 1999 that were too small to mark, escaped capture, or were marked or recaptured (% recaptured in parentheses).

Species	Stream	Not marked	Escaped	Marked	Recaptured (%)
Desmognathus	Jeremy's Run	24	57	56	15 (27)
	Keyser Run	14	29	35	1 (3)
	Land's Run	32	30	28	7 (25)
	North Fork Thornton	15	20	34	2 (6)
	Pass Run Tributary	14	25	18	2 (11)
	Piney River	8	12	9	1 (11)
Eurycea bislineata	Jeremy's Run	7	14	3	1 (33)
	Keyser Run	4	2	2	0 (0)
	Land's Run	4	4	5	0 (0)
	North Fork Thornton	12	20	35	0 (0)
	Pass Run Tributary	12	12	14	0 (0)
	Piney River	3	7	3	0 (0)
Gyrinophilus / Pseudotriton	Jeremy's Run	0	7	8	1 (13)
	Keyser Run	0	1	1	0 (0)
	Land's Run	0	3	1	0 (0)
	North Fork Thornton	0	1	2	0 (0)
	Pass Run Tributary	0	0	1	0 (0)
	Piney River	0	5	4	0 (0)

Table 2. – Population estimates ($N \pm$ standard error, s_x), 95 % confidence intervals (CI), detection probabilities ($\hat{p} \pm s_x$) and models used for species encountered during capture-recapture (CR) and removal (REM) sampling in 1999 at 6 streams in Shenandoah National Park. For Bayesian results, we present the mean $\bar{N}$ /mode $\hat{N}$.

Species - Stream	Method	n	Passes	$\hat{N}\ (s_x)$	95 % CI	$\hat{p}\ (s_x)$	Model
Desmognathus							
Jeremy's Run	CR	6		116 (22.5)	86-178	0.10 (0.023)	O
	Bayesian	6		124/119 (25.9)	86-185	0.10 (0.023)	
	REM	6	29,11,13,12,8,7	97 (10.4)	86-131	0.25 (0.053)	B
Land's Run	CR	6		62 (18.8)	41-122	0.09 (0.032)	O
	Bayesian	6		72/66 (25.6)	41-137	0.09 (0.030)	
	REM	6	9,6,5,8,5,4	63 (27.6)	42-180	0.14 (0.085)	B
Pass Run Tributary	CR	5		71 (44.9)	31-242	0.06 (0.038)	O
	Bayesian	5		115/84 (104.9)	32-392	0.06 (0.035)	
	REM	6	12,5,6,6,5,9	108 (90.0)	52-537	0.08 (0.083)	B
Piney River	CR	5		33 (29.4)	13-166	0.06 (0.056)	O
	Bayesian	5		95/47 (159.4)	13-494	0.06 (0.048)	
	REM	6	5,1,4,4,3,0	21 (5.6)	18-48	0.23 (0.117)	B
Keyser Run	CR	5		493 (479.2)	120-2505	0.02 (0.014)	O
	Bayesian	5		556/419 (461.4)	128-1844	0.02 (0.015)	
	REM	6	9,13,1,0,0,0	23 (0.0)	23-23	0.61 (0.079)	B
North Fork Thornton	CR	6		253 (169.3)	91-872	0.02 (0.016)	O
	Bayesian	6		346/266 (275.5)	97-1074	0.03 (0.016)	
	REM	6	11,14,2,11,4,6	70 (18.3)	53-139	0.17 (0.072)	B
Eurycea bislineata							
Jeremy's Run	CR	6		4 (2.7)	3-20	0.17 (0.139)	O
	Bayesian	6		19/7 (60.1)	3-110	0.12 (0.095)	
	REM	6	6,2,2,5,4,3	50 (51.3)	25-323	0.09 (0.115)	B
Pass Run	REM	6	1,2,0,3,0,0	6 (1.1)	6-6	0.35 (0.116)	B
Piney River	REM	6	1,0,0,1,1,0	3 (0.0)	3-3	0.30 (0.145)	B
Gyrinophilus Pseudotriton							
Jeremy's Run	CR	6		27 (24.2)	11-137	0.06 (0.051)	O
	Bayesian	6		82/40 (138.4)	11-433	0.05 (0.043)	
North Fork Thornton	REM	6	0,1,0,0,0,0	1 (0.0)	1-1	0.50 (0.354)	B
Piney River	REM	6	1,0,1,1,0,0	3 (0.0)	3-3	0.38 (0.171)	B

Table 3. Population estimates ($\hat{N} \pm s_x$), 95 % confidence intervals (CI) and detection probabilities ($\hat{p} \pm s_x$) for salamanders encountered during removal sampling in Shenandoah National Park. Analyses were based on two or three diurnal passes conducted consecutively at one (2001) or two (2002-04) transects (T) at each of eight streams (escaped salamanders excluded) using model M_B (Zippin) from program CAPTURE. – indicates a third pass was not conducted. No data for a particular species for a year, stream or transect indicates that none were detected or that estimation was not possible.

Taxon - Stream	Year	T	Passes 1,2,3	$\hat{N}$ (s_x)	95% CI	$\hat{p}$ (s_x)
Desmognathus						
Piney River	2004	1	1,1,0	2 (0.38)	2-2	0.67 (0.272)
Piney Tributary	2002	1	3,0,1	4 (0.54)	4-4	0.67 (0.192)
		2	4,2,2	9 (1.86)	9-20	0.52 (0.245)
	2003	1	2,1,-	3 (0.75)	3-3	0.75 (0.217)
	2004	1	2,1,0	3 (0.27)	3-3	0.75 (0.217)
		2	2,1,0	3 (0.27)	3-3	0.75 (0.217)
Ivy Creek	2001	1	19,12,-	44 (14.98)	34-109	0.45 (0.207)
	2002	1	31,4,-	35 (0.75)	35-35	0.90 (0.049)
		2	29,12,-	47 (5.80)	43-70	0.63 (0.129)
	2003	1	6,4,-	12 (3.85)	11-34	0.58 (0.297)
		2	1,1,0	2 (0.38)	2-2	0.67 (0.272)
	2004	1	3,3,2	10 (4.30)	9-34	0.39 (0.285)
		2	7,0,2	9 (0.69)	9-9	0.69 (0.128)
Doyle's River	2001	1	9,1,-	10 (0.35)	10-10	0.91 (0.087)
	2002	2	7,5,0	12 (0.73)	12-12	0.71 (0.111)
	2004	1	5,1,2	8 (1.06)	8-8	0.62 (0.135)
Hawksbill	2001	1	11,8,-	28 (14.37)	21-100	0.42 (0.278)
	2002	1	24,1,-	25 (0.21)	25-25	0.96 (0.038)
	2003	2	1,2,0	3 (0.71)	3-3	0.60 (0.219)
	2004	1	2,0,1	3 (0.71)	3-3	0.60 (0.219)
		2	6,1,1	8 (0.51)	8-8	0.73 (0.134)
Pass Run	2002	2	6,1,2	9 (0.95)	9-9	0.64 (0.128
	2004	1	3,4,0	7 (0.87)	7-7	0.64 (0.145)
		2	4,2,1	7 (0.87)	7-7	0.64 (0.145)
Jeremy's Run	2001	1	8,4,-	13 (2.41)	13-27	0.68 (0.224
	2002	1	9,4,4	20 (4.15)	18-40	0.46 (0.177)
		2	4,2,0	6 (0.38)	6-6	0.75 (0.153)
	2003	1	13,3,-	16 (0.88)	16-16	0.84 (0.084)
		2	7,5,-	15 (6.61)	13-51	0.51 (0.307)
	2004	1	10,6,0	16 (0.72)	16-16	0.73 (0.095)
		2	7,2,1	10 (0.63)	10-10	0.71 (0.121)
Eurycea						
Piney River	2002	2	10,6,1	17 (1.04)	17-22	0.68 (0.13[illegible])
	2003	2	1,1,0	2 (0.38)	2-2	0.66 (0.272
	2004	1	5,4,2	12 (2.74)	12-28	0.49 (0.215)
Piney Tributary	2001	1	14,7,-	24 (4.97)	22-48	0.60 (0.193)
	2002	2	8,11,4	34 (14.05)	25-98	0.31 (0.183)
	2003	1	17,6,-	24 (2.47)	24-37	0.73 (0.141)
		2	12,7,-	24 (6.77)	20-56	0.54 (0.228)
	2004	1	9,1,2	12 (0.73)	12-12	0.71 (0.111)
		2	5,4,1	10 (1.11)	10-10	0.62 (0.121)
Ivy Creek	2001	1	7,1,-	8 (0.40)	8-8	0.89 (0.105)
Doyle's River	2003	1	4,2,-	6 (1.05)	6-6	0.75 (0.153)
	2004	1	6,3,1	10 (0.86)	10-10	0.67 (0.122)
Hawksbill	2001	1	6,3,-	9 (1.43)	9-18	0.74 (0.232)
	2002	1	18,4,-	22 (1.00)	22-22	0.85 (0.07[illegible])
	2003	1	4,2,-	6 (1.05)	6-6	0.75 (0.153)
	2004	1	3,1,2	6 (1.69)	6-17	0.51 (0.294)
		2	18,2,1	21 (0.32)	21-21	0.84 (0.073)
Pass Run	2002	2	12,8,4	28 (4.59)	25-48	0.47 (0.146)
	2004	1	10,4,6	27 (9.65)	21-72	0.35 (0.187)
		2	14,9,7	40 (11.15)	32-87	0.36 (0.15[illegible])
Jeremy's Run	2001	1	6,2,-	8 (0.87)	8-8	0.80 (0.177)
	2002	1	5,1,2	8 (1.06)	8-8	0.62 (0.135)
	2004	1	12,3,3	18 (1.32)	18-27	0.65 (0.13[illegible])
		2	5,2,0	7 (0.33)	7-7	0.78 (0.139
Paine Run	2001	1	29,23,-	112 (74.11)	62-447	0.27 (0.206
	2002	1	16,9,-	32 (8.27)	26-69	0.53 (0.202
		2	117,21,-	146 (3.56)	143-158	0.81 (0.044
	2003	2	21,9	34 (4.84)	31-55	0.64 (0.15[illegible]
	2004	1	5,[illegible],0	6 (0.14)	6-6	0.86 (0.132
		2	3,3,0	6 (0.67)	6-6	0.67 (0.157
Gyrinophilus [illegible]						
Ivy Creek	2002	2	4,2,-	6 (1.05)	6-120	0.75 (0.153)
Doyle's River	2002	1	6,7,4	27 (16.50)	19-[illegible]10	0.27 (0.[illegible])
		2	5,1,1	6 (0.38)	6-6	0.75 (0.15[illegible])
Jeremy's Run	2004	1	2,2,0	4 (0.54)	4-4	0.67 (0.[illegible]2
Paine Run	2002	1	3,2,-	5 (1.20)	5-5	0.71 (0.17[illegible])
	2004	1	10,5,3	19 (2.50)	19-32	0.54 (0.154)
		2	2,2,0	4 (0.54)	4-4	0.67 (0.[illegible]92)

To analyze population change at a site, at least two years of data are needed and analyses should rely on population estimates to avoid bias associated with raw counts. For *Desmognathus* and *Eurycea*, we had complete sets of $\hat{N}$ for 2001-2004 at three streams each and $\hat{N}$ for 3 of the 4 years at another two streams (tab. 3). We found significant differences in $\hat{N}$ across years ($F = 12.7$, $df = 3,9$, $P = 0.001$) and streams ($F = 9.9$, $df = 4,9$, $P = 0.002$) as well as a significant year*stream interaction ($F = 4.6$, $df = 10,9$, $P = 0.015$) for *Desmognathus*, but no significant differences for *Eurycea*. *Desmognathus* population estimates were significantly higher in 2001 (24 ± 7.8) and 2002 (20 ± 5.4) compared to 2003 (9 ± 2.7) and 2004 (8 ± 1.4). The park experienced heavy precipitation during the summer of 2003, with average stream flow rates 19 and 7 times higher than stream flow rates in the summers of 2002 and 2001, respectively (Shenandoah Watershed Study data, Rick Webb, pers. comm.). Data from July 2004 are not yet available, but 2004 flow rates were most likely intermediate between the 2003 and 2001-2002 flow rates.

DISCUSSION

Long-term monitoring programs require cost-effective and efficient techniques to gather accurate and precise data. Unfortunately, the spatially variable (i.e., significant differences among streams) and sometimes low detection probabilities found in this study using capture-recapture and removal methods reinforce the need for estimating $\hat{p}$'s as part of stream salamander abundance estimation studies. Our study also indicates the importance of developing better methods for estimating stream salamander populations such that estimates are consistently available on a yearly basis for trend analyses.

We found that removal sampling yielded higher $\hat{p}$'s for stream salamanders than capture-recapture sampling. Other capture-recapture surveys of stream salamanders have also shown low recapture rates and hence detection probabilities (BARTHALMUS & BELLIS, 1972; NIJHUIS & KAPLAN, 1998). Indeed, MAROLD (2001) used VIE to mark 44 *E. bislineata* and *D. fuscus* but did not recapture any in the field. BRUCE (1995) used removal sampling (7 passes set 2-3 days apart) and found low to moderate standard errors for population estimates of *D. monticola* and suggested removal sampling was a promising technique to monitor salamander demographics. Other factors favoring removal over capture recapture sampling are that removal sampling usually requires shorter sampling intervals, reduced field personnel, and less funding than capture-recapture, and appears to be ideal for amphibians such as aquatic larvae that are highly detectable and have limited home ranges and mobility (HAYEK, 1994).

If removal sampling is to be used for long-term monitoring, field protocols play an important role in determining their success. In our removal surveys, effective reduction of populations sometimes did not occur even after six passes. This may be due in part to the high percentage of salamanders that escaped capture, though if we analyzed removal data including escapes in the passes, the same issues would be apparent. It is important to note that when the percent of escapes were lower as they were in 2001-2004 compared to 1999, the $\hat{p}$'s for species were higher. With fewer escapes and larger sample sizes, there is potential for better estimates. Removal estimates using Zippin's method are unreliable if less than half the population is removed (BRUCE, 1995), but WHITE et al. (1982) considered detection probabilities greater than 0.20 adequate for estimating population abundance in removal experiments. BRUCE (1995) found that 7 passes probably reduced total *D. monticola* populations by more than half at his study sites, but he had difficulties reducing numbers of first year juveniles, which may have shown "increased surface activity...as the larger salamanders were removed (i.e., a response to reduced competition or predation)". SOUTHERLAND et al. (2004) used two-pass removal sampling and were unable to calculate population estimates for species at an average of 75 % of the streams surveyed because salamander numbers did not decrease or were zero in the second pass. Removing salamanders from under the top layer of rocks may disturb or "unearth" other salamanders deeper in the rock substrate. As we sometimes observed, this can lead to more salamanders in the surface population during subsequent passes than in the first pass before disturbance.

Several factors could be changed in our removal protocol to improve $\hat{N}$ and $\hat{p}$ estimates. Conducting surveys on wet or humid nights, when more of the salamander population may be on the surface foraging, might yield better removal estimates. *D. fuscus* and *E. bislineata* emerge one hour after sunset (HOLOMUZKI, 1980) and *D. monticola* emerge shortly after dark, with peak activity occurring around midnight and again at dawn (SHEALY, 1975; HAIRSTON, 1986). However, working at night along rocky streams can be difficult and treacherous. Another option would be to conduct more removal passes, providing the option to group data from earlier passes in which no decreases in removals occurred. Pilot studies in which a large number of removal passes are conducted to determine the appropriate number and grouping of passes may be useful. Another factor to consider is the size and placement of transects or plots. In our surveys, we only searched narrow 1- or 2-m bands along and/or in the stream. Most stream salamanders move between the stream channel, splash zone and bank. Home ranges of *D. fuscus* have been shown to vary tremendously, from 1.4 m^2 in Ohio (ASHTON, 1975) to 25-114 m^2 in Kentucky (BARBOUR et al., 1969). *D. monticola* home ranges were estimated to be 8.4 m^2 in Kentucky (HARDIN et al., 1969). During warm months, *E. bislineata* tagged with radioactive isotopes moved within a 14 m^2 area (ASHTON & ASHTON, 1978), but in June some post-breeding migrants moved more than 100 m from a stream (MACCULLOCH & BIDER, 1975), which probably explains the particularly low recaptures we observed for this species in the 1999 capture-recapture surveys. Surveying a wider area of bank along with the stream channel to incorporate more of the target species' individual home ranges may yield better removal estimates.

Other new approaches may prove to be more useful for stream salamander population estimation. Our removal estimates were based on populations at single stream transects. New analytical methods developed by ROYLE (2004*a-b*), ROYLE et al. (2004) and DORAZIO et al. (in press) aggregate information across sample sites such that removal sampling can estimate the abundance of spatially distinct subpopulations. These models incorporate spatial models of abundance (e.g., Poisson, negative binomial) with models of detection probability and have been shown to yield abundance estimates with "similar or better precision than those computed using the conventional approach of analyzing the removal counts of each subpopulation separately" (DORAZIO et al., in press).

A different approach would be to estimate the proportion of area (in this case, streams) occupied (PAO) by stream salamanders over time (MACKENZIE et al., 2002). The PAO method estimates site occupancy and detectability of species based on presence/absence data recorded from repeated visits to sites selected using a probabilistic sampling frame within an area of inference. Stream salamander species that exhibit low detection probabilities and occupy fewer sites would require more streams and visits per stream for PAO estimation (MACKENZIE & ROYLE, submitted). Note that repeated visits to streams could be satisfied by surveying multiple transects along the length of a stream.

Despite the problems evident in this study, population estimation efforts incorporating detection probabilities may be necessary to assess trends in stream salamander populations. Better survey methods (e.g., transect designs) and population estimation techniques (e.g., aggregated removal or PAO approaches) need to be tested and developed such that reasonably low bias population estimates can be consistently calculated for sites over time. In addition, spatial design of sampling associated with hypothesis testing incorporating covariates that may influence stream salamanders ($\hat{p}$, $\hat{N}$, site occupancy), such as the percent of impervious surface in a watershed and stream flow rates, should be incorporated alongside monitoring to best yield inferences about how changes in stream salamander populations over time are influenced by environmental factors.

RÉSUMÉ

Dans l'est des États-Unis, les salamandres torrenticoles de la famille des Plethodontidae représentent une biomasse élevée dans et auprès des ruisseaux issus des sources. Elles peuvent ainsi constituer d'intéressants indicateurs de la santé de ces écosystèmes. Beaucoup d'etudes de ces salamandres se sont appuyees sur des indices démographiques utilisant des décomptes d'animaux et non pas sur des estimations fondées sur des techniques comme les captures-recaptures ou le ramassage des individus. L'emploi de procédures d'evaluation permet le calcul de probabilités de détection (la proportion d'animaux réellement presents détectés lors d'une etude) et de leur ecart-type, et peut permettre de déterminer les tailles et les dynamiques des populations de salamandres. En 1999, nous avons employé les méthodes de capture-recapture et de ramassage pour évaluer des populations de salamandres du genre *Desmognathus* dans six ruisseaux du Parc National de Shenandoah (Virginie, Etats-Unis). La méthode du ramassage s'est avérée plus efficace: elle a donné des probabilites de détection plus élevees que celle de capture-recapture. Pendant la période 2001-2004, nous avons employé la methode du ramassage dans et auprès de huit ruisseaux du Parc afin d'évaluer la fiabilite de cette technique pour la surveillance à long terme de ces populations de salamandres. Lors de transects le long des ruisseaux, nous avons obtenu des probabilites de detection de 0,39 à 0,96 pour *Desmognathus*, de 0,27 à 0,89 pour *Eurycea* et de 0,27 à 0,75 pour *Gyrinophilus porphyriticus*/*Pseudotriton ruber*. Les probabilités de détection n'ont pas varié au cours des annees pour *Desmognathus* et *Eurycea*, mais ont différé selon les ruisseaux pour *Desmognathus*. Les evaluations des populations de *Desmognathus* ont diminue entre 2001-2002 et 2003-2004, ce qui peut être lié à des changements dans le régime hydrique des ruisseaux. Les procedures de ramassage constituent une methode fiable pour l'evaluation de populations de ces salamandres, mais les méthodes de terrain doivent être conçues de manière à remplir les conditions statistiques des méthodes d'échantillonnage. De nouvelles méthodes d'estimation des populations de ces salamandres sont discutées.

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